

Extraction of Carotenoid Extracts from Kumquat Waste by Polarity Solvent and Investigation of Its antioxidant Power

Fatemeh Shooshtari and Vahid Hakimzadeh *

^{1,2} Department of Food Science and Technology, Quchan Branch
Islamic Azad University, Quchan, Iran

*Corresponding author's email: v.hakimzadeh [AT] yahoo.com

ABSTRACT— Carotenoids can be used as nutritional supplements, pigments, and natural antioxidants in food industry. Carotenoids extant in fruits and vegetables are recognised as an effective antioxidant in various nutritional systems. The objective of this study was to investigate the effects of different polarity solvent on the carotenoid contents of Kumquat fruit wastes. Solvents such as methanol, ethyl acetate, and ethanol at 25 and 45°C were employed in the extraction process. The antioxidant activity of these extracts was investigated by the widely used DPPH, FRAP, total phenol method and the β -carotene linoleic acid test. The results based on antioxidant power indicated that the most suitable condition for extraction of carotenoids was the use of ethyl acetate solvent at 40°C.

Keywords--- Antioxidant, Carotenoid, Ethyl Acetate, Kumquat, Total Phenol

1. INTRODUCTION

This investigation therefore concentrates upon the extraction of carotenoid from kumquat wastes and determination of its antioxidant power. Kumquat botanically belongs to the *Rutaceae* Family of the *Fortunella* Genus. The fruit when sliced has the appearance of a small orange whose segments are connected to each other and the external rind while one or two seeds can generally be seen in each segment. This plant has many varieties though only 4 are cultivated for their fruits. Cultivation of the common varieties of kumquat only takes place in tropical regions and the tree is in semi-active and semi-hibernation state in subtropical regions and during the autumn, winter, and spring months [Advali *et al.*, 2011].

Waste management has nowadays assumed great importance and awareness of the cost effectiveness of treatment of waste from various industries such as food is on the rise. To that end one of the most extensive research areas is the extraction of vital and ultra –beneficial compounds such as antioxidants, enzymes, antimicrobial compounds etc. from these seemingly unusable substances for use in other areas of the food industry [Delgado-vargas *et al.*,2003;Ranjbar *et al.*,2010]. Carotenoids meanwhile have huge applications in various foodstuffs due to their wide distribution among animal and plant sources and their nutritional and antioxidant property [Socaciu *et al.*, 2008].

Carotenoids are synthesized only by plants and microorganisms as humans and animals cannot perform this action. They produce a yellow, orange, and Red colour spectrum by absorbing visible light. Not only do carotenoids produce colour and Vitamin A precursors but they also have antioxidant properties.

Fruits and vegetables are two major producers of carotenoids in diets. Alpha –Carotene, Beta – Carotene, Beta – Kryptoxanthin, Lutein, Zeaxanthin, and Lycopene are among the most common carotenoids [Abrahamsson *et al.*, 2012; A.V.Rao *et al.*, 2007; Delia *et al.*, 2004;Hsin-Lan *et al.*, 2014; Socaciu.,2008]. All carotenoids play a role in human health due to their antioxidant properties and are effective in the prevention of cancer [Delia *et al.*, 2004; Hopkins *et al.*, 1999; Khonsaran *et al.*, 2012; Roberts *et al.*, 2009; Michaud *et al.*, 2000; Moden *et al.*, 1986; Perkins *et al.*, 2001]. Antioxidant activity of carotenoids is due to the existence of conjugated double bonds in end groups (looped or otherwise), and active inter-loop groups [Perkins *et al.*, 2001]. It should however be noted that variety in the structure of carotenoids results in variety in their performance such that the antioxidant power of three types of carotenoid can be expressed thus: Lycopene > β -carotene> α -carotene [Anguelova *et al.*, 2000; Mehdizadeh *et al.*, 2009]. Roozy *et al.*, (2002) were able to separate various carotene and tocopherol isomers from tomato peel, thus indicating not only a solution to rid the environment of the danger of waste pollution but also due to the high cost of natural pigments and antioxidants make production in agricultural and food areas more cost effective.

2. MATERIALS & METHODS

In the present research three solvents ethanol, methanol, and ethyl acetate were applied at 25 and 45°C, respectively so as to extract the carotenoids from kumquat waste. The antioxidant power of all samples were evaluated by the Total Phenol, Beta-carotene Linoleic Acid, FRAP, and DPPH assays.

Extract Preparation

Kumquat samples were purchase from citrus groves in the city of Ramsar. Fruits were peeled following washing and the peels dried in the German Memmert Vacuum oven at 40°C for 12 hours at a vacuum pressure of 70 mbar. Dried peels were then powdered by the German Pulverisette 14 Mill and preserved in a dry cool place. Peel powder (15g) and 150 ml of each solvent (10:1 ratio) were poured in an erlen containing a lid and placed in shaker incubator at 25 and 40°C for each solvent at 150 rpm for 12 hours so as to ensure the complete dissolution of the powder in the solvent. The resultant solution was finally sifted through filter paper and then placed in a German Vacuum Rotary Operator at 40°C and 70 rpm for 50 minutes. The final solution for further assurance was sifted through filter paper and the Buchner Funnel and the extract obtained was refrigerated at 4°C.

Determination of total phenolic content

Total phenolic content was evaluated in the methanol extracts, according to the Folin-Ciocalteu method with slight modifications [Hayouni et al., 2007]. One ml aliquot of the extract was mixed with 5 ml of Folin-Ciocalteu phenol reagent (diluted with water 1:10 v/v) and 4 ml of sodium carbonate (75 g/ L). The tubes were vortexed for 30 s and allowed to stand for 60 min at room temperature (35 ± 2°C) for colour development. The absorbance was measured at 765 nm by spectrophotometer. A calibration curve ($R^2 = 9858$) of Gallic acid (0-0.10 mg/ ml) was prepared and tested under similar conditions. The results were expressed as mg of Gallic acid equivalents per 100 g of dry weight (mg GAE/ 100 g DW). All samples were analysed trice and the results averaged.

DPPH free radical scavenging assay

The antioxidant activity of the extract was measured by DPPH assay described by Ravichandran et al. [2013] as follows: 0.1 ml of the methanol extracts was mixed for 30 s with 3.9 ml of DPPH solution (6 x 10⁻⁵ M). The solution was incubated at room temperature for 30 min, and the decrease in absorbance at 518 nm was measured at the end of incubation period with a spectrophotometer. The DPPH solution without extract was analysed as control. The antioxidant activity was calculated as equation 1.

$$\text{Equation 1: } I\% = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} * 100$$

Where:

A_{control} = Absorption of the control sample at 518nm

A_{sample} = Absorption of sample at 518nm

Ferric Reducing Antioxidant Power Assay (FRAP Assay)

This method is based on the reduction of Fe³⁺ to Fe²⁺, which is recorded by measuring the formation of a blue coloured Fe²⁺-Tripyridyltriazine compound from the colourless oxidized Fe³⁺ form via the action of electron-donating antioxidants. The FRAP reagent consists of 300 mM acetate buffer (3.1 g sodium acetate + 16 mL glacial acetic acid, made up to 1 litre with distilled water; pH = 3.6), 10 mM TPTZ in 40 mM HCl and 20 mM FeCl₃.6H₂O in a ratio of 10:1:1. The extract (50 μL) was added to 1.5 mL freshly prepared and pre-warmed (37 °C) FRAP reagent. The mixture was incubated at 37 °C for 10 min and the absorbance was measured against a reagent blank (1.5 mL FRAP reagent + 50 μL distilled water) at 593 nm. A standard curve of Fe (II) was constructed over the concentration range of 0.1 mM to 2.0 mM. The results were determined by the regression equation of the curve ($r^2 = 0.9485$) and expressed as μ mole ferric ions reduced to ferrous form. [Benzie *et al.*, 1999].

β -Carotene-linoleic acid assay

This test was evaluated using β - carotene/linoleic acid model system, as described by Miller (1971). Two ml of a solution of -carotene in chloroform (1 mg/ml) was pipetted into a flask containing 40 mg of linoleic acid and 400 mg of Tween 40. The chloroform was removed by rotary vacuum evaporator at 45°C for 4 min, and 100 ml of distilled water was added slowly to the semisolid residue with vigorous agitation to form an emulsion. A 5 ml aliquot of the emulsion was added to the tubes containing 0.2 ml of various concentrations (0.125 to 1.0 mg/ml) of sample, and the absorbance was measured at 470 nm, immediately, against a blank, consisting of the emulsion without β - carotene. The tubes were placed in a water bath at 50°C, and the absorbance was measured again at 120 min. The antioxidant activity (AAI) was calculated as percentage of inhibition relative to the control using the equation 2.

$$\text{Equation 2: } AAI\% = \frac{[1-(A_{initial}-A_t)]Sample}{(A_{initial}-A_t)control}$$

Where:

A_{initial} = Initial absorption reading at 470nm

A_t = Absorption reading after 120 minutes at 470nm

Statistical design and analysis

Compared means of data calculated by Tucky method in SPSS software and graphs were plotted by excel.

3. RESULTS

Total Phenol Content

Table 1 shows the Total Phenol levels in different extracting solvents of kumquat waste. The results indicated that carotenoid extracted at 40°C and containing the reagent Ethyl Acetate had the greatest phenol compounds content which is indicative of its high antioxidant power. This can be due to the lower polarity of ethyl acetate in comparison to methanol and ethanol as phenol compounds are low polarity voluminous compounds [Mohamadi *et al.*, 2012].

Jayaprakhasha *et al* (2003) reported that ethyl acetate was successful in the extraction of phenol compounds such as Catechins and Epicatechins from the grape. Tests carried out by Amir Salari *et al* (2008) also indicated that solvents containing a higher proportion of ethyl acetate are more effective in the extraction of the phenol compounds in the grape.

Table 1: Total Phenol content in the Ethanol, Methanol, and Ethyl Acetate Carotenoid Extracts Resultant from Kumquat waste

Type of Solvent	Temperature (°C)	Total Phenolic (mg GAE /g)
Ethanol	25	0.013 d
Ethanol	40	0.06 c
Methanol	25	0.126 b
Methanol	40	0.125 b
Ethyl Acetate	25	0.146 b
Ethyl Acetate	40	0.2 a

Radical Scavenging Activity (RSA) on DPPH and Ferric Reducing Antioxidant Power (FRAP)

The results indicated that the carotenoid extracted through the solvent ethyl acetate had a higher percentage of deterrence than those extracted involving other solvents. No significant difference was noticed between the deterrence percentage of ethyl acetate at 25 and 40°C, However, the carotenoid extracted at 40°C had a higher ability to scavenge the free radicals. The percentage of free radicals scavenged can be seen in Table 2:

Table 2: The Scavenging Extent of Free Radicals in Kumquat Waste extracted by using the Ethanol, Methanol, and Ethyl Acetate Carotenoid Extracts Waste

Type of Solvent	Temperature (°C)	Scavenging percentage (I %)
Ethanol	25	34.85 d
Ethanol	40	37.87 c
Methanol	25	34.40 d
Methanol	40	41.80 b
Ethyl Acetate	25	49.52 a
Ethyl Acetate	40	50.18 a

Reductive Power

The evaluation of the regenerative power of the extracts in μmole Fe II/lit indicated that the extract produced from ethyl acetate at 40°C again had the highest regenerative power among all samples (Table 3).

Table 3: Reductive Power of Iron in Conjunction with Ethanol, Methanol, and Ethyl Acetate Carotenoid Extracts in Kumquat Waste

Type of Solvent	Temperature (°C)	x (µmole Fe/lit)
Ethanol	25	1.39 e
Ethanol	40	1.46 d
Methanol	25	1.50 c
Methanol	40	1.52 c
Ethyl Acetate	25	1.91 b
Ethyl Acetate	40	2.44 a

Beta – Carotene Linoleic Acid

Antioxidant percentage of power determined from equation 2 can be seen in Table 4. The carotenoid extracts resultant from the ethyl acetate solvent had the highest ability in scavenging oxidation of linoleic acid. Difference was that the extract produced at 25°C indicated a slightly higher antioxidant power than the 40°C sample.

Table 4: Oxidative Scavenging Extent of Linoleic Acid in Conjunction with Ethanol, Methanol, and Ethyl Acetate Carotenoid Extracts evaluated from Kumquat Waste

Type of Solvent	Temperature (°C)	AAI%
Ethanol	25	20.81 e
Ethanol	40	21.04 e
Methanol	25	38.84 b
Methanol	40	35.07 c
Ethyl Acetate	25	40.40 a
Ethyl Acetate	40	33.25 d

4. DISCUSSION

Overall comparison of the peel and the flesh indicated higher content phenol and flavonoid compounds in the peel than in the flesh of the fruit. The higher quantity of phenol compounds in the peel is due to the accumulation of these same compounds in the epidermal tissue of the plant because of their responsibility for the protection of the plant against ultraviolet radiation, insects and diseases [Dixon *et al.*, 1995; Qarakhani *et al.*, 2010]. During the study it was found that the tomato peel had a higher phenol and flavonoid content than its flesh [Qarakhani *et al.*, 2010; Toor *et al.*, 2005].

Plants contain a variety of compounds with different structures. Extraction of these compounds is dependent upon many factors of which solvent and method of extraction are the most important. The choice of solvent and method of extraction is dependent upon the different parts of a plant and its composition. It would be extremely difficult to allocate a special solvent for each group of botanical compounds as other substances extant alongside these compounds tend to affect the degree of solvency [Haghipoor *et al.*, 2009; Islam *et al.*, 2015; Samsam shariat *et al.*, 1992].

Fayazmehr *et al.*, (2012) investigated the effect of ultrasound waves on the antioxidant content and capacity of tomato bagasse using ethyl acetate and hexane solvents in conjunction with the current research and found that the extract resultant from ethyl acetate had the highest antioxidant capacity. This suggested a higher influence of ethyl acetate than hexane. Research by Sheikhzadeh *et al* (2015) on the extraction of carotenoids exhibited that banana skin in ethyl acetate had the most iron regenerative quality in comparison to ethanol and methanol.

Hosseini *et al.*, (2011) investigated the capability of the essence and different groups of the methanol extracts of thyme, sage, rosemary, penny royal, and cinnamon in scavenging free radicals by using various solvents such as methanol, hexane, ethyl acetate, and dichloromethane and reported that ethyl acetate had the highest antioxidant effect, which is due to the polarity of the antioxidant compounds.

Fikselova *et al* (2008) reported 5.68 mg/100 g carotene extracted at 60°C which in comparison to the initial amount of 4.12 mg/100 g at 40°C showed an increase. Their results indicated that the increase in temperature to 60°C has a positive effect on the efficiency of carotenoid extraction. Results endorse the results of the present research on the higher antioxidant power of the ethyl acetate extract at 40°C.

5. CONCLUSION

As can be seen, Kumquat waste can be considered as a useful source of phenolic compounds and carotenoids. For extraction of these components Ethyl acetate was better performance compared to Methanol and Ethanol. Also, extracts obtained in higher temperature (40°C) effectiveness than lower temperature (25°C) in antioxidant activity and total phenolic component, but beta carotene method that was slightly better at 25°C in antioxidant activity. Quantity of total phenols in ethyl acetate extract at 40°C was 0.2 mg/g. Ability of Scavenge DPPH radicals for ethyl acetate extracts at 40°C obtained 50.18 based on I%, too. Also, reductive power for ethyl acetate extract at 40°C was calculated 2.44 µmol FeII/lit. But, the best of temperature due to extraction of antioxidant components by ethyl acetate in scavenging oxidation of linoleic acid was 25°C that determinate 40.40 AAI%.

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